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14. ABSTRACT Although the combination of radiation therapy and androgen deprivation therapy (ADT) is initially effective in many patients, biochemical failure rates of 20% at 5-years to 50% at 10-years have been reported, highlighting the need for improved treatments, particularly for men with high-risk prostate cancer. ADT in the neo-adjuvant setting is used to reduce tumor volume and improve the response to radiation. Additionally, ADT causes infiltration of lymphocytes into the prostate. B cell infiltrates may promote prostate cancer progression and development of castration resistant prostate cancer by the production of inflammatory cytokines and skewing CD4+ T cell responses towards Th2. We hypothesized that depletion of B cells at the time of castration would improve tumor control. Our results demonstrate that the depletion of B cells at the time of castration improves tumor latency.					
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Introduction

It is becoming increasingly clear that many standard cancer treatments trigger anti-tumor immune responses that influence tumor control. The nature and magnitude of these responses vary depending on the type of tumor and treatment modality. In prostate cancer, we have previously reported that radiation and androgen deprivation therapy (ADT) induce tumor associated autoantibody responses (1, 2). ADT is thought to increase tumor cytorreduction and apoptosis while decreasing tumor hypoxia, which may enhance the tumoricidal effects of radiation (3). A central component of the humoral immune responses is antibody production by B cells, which is dependent on Th2 cytokines via CD4 help. Th2-associated cytokines may stimulate angiogenesis, blunt CTL responses, and initiate chronic inflammation in the tumor environment, ultimately leading to poor outcomes (4, 5). Studies have shown that a tumor specific humoral immune response is induced by ADT, radiation or vaccine approaches in some prostate cancer patients (6-8). Thus, current treatments for prostate cancer may induce detrimental immune responses in some patients. It may be possible to improve outcomes by administering immune modulatory agents during hormone and radiation therapy with the goal of diverting tumor immunity away from humoral immunity toward a more beneficial cytolytic response. Preliminary data in the Shionogi tumor model suggested that CD4⁺ depletion is helpful in preventing tumor recurrence and that this correlated with a lack of antibodies against the protein PABN1. We hypothesized that directly depleting B cells would improve tumor control. Our results indicate that B cell depletion improves tumor control after castration in the TRAMP-C2 mouse model. These results suggest that the combination of ADT and B cell depletion may improve tumor control in prostate cancer patients during initial treatment and ultimately prevent recurrence.

Body:

Task 1. To determine if CD4⁺ T cell depletion delays tumor progression using TRAMP-C2 tumors.

Determine if TRAMP-C2 tumors induce an antibody response in castrated mice.

Mice were implanted with 5×10^6 TRAMP-C2 tumors in the flank and were allowed to establish for 30 days before castration. Blood was taken 1 week prior to castration and then 3 and 5 weeks after castration. TRAMP-C2 were lysed at $\sim 1 \times 10^6$ cells/mL in radioimmunoprecipitation assay buffer [50 mmol/L Tris-HCl (pH 7.5); 150 mmol/L NaCl; 1% NP40; 0.5% sodium deoxycholate; 0.1% SDS] on ice for 30 min. After centrifugation, 400 µg of protein were separated using NuPAGE Novex 4% to 12% Bis-Tris gels (Invitrogen, Burlington, ON, Canada) and transferred to nitrocellulose using the XCell SureLock Mini-Cell (Invitrogen). Sera were diluted 1/500 in Blotto (5% dry milk powder; 0.1% Tween 20; 50 mmol/L Tris; 150 mmol/L NaCl) and incubated with nitrocellulose membranes for 1 h at room temperature using a multichannel immuoblotting device (Mini Protean II Multiscreen, Bio-Rad, Mississauga, ON, Canada). The membrane was then incubated for 1 h at room temperature with IRDye700 goat anti-mouse IgG (H+L) and visualized on a Licor Odyssey.

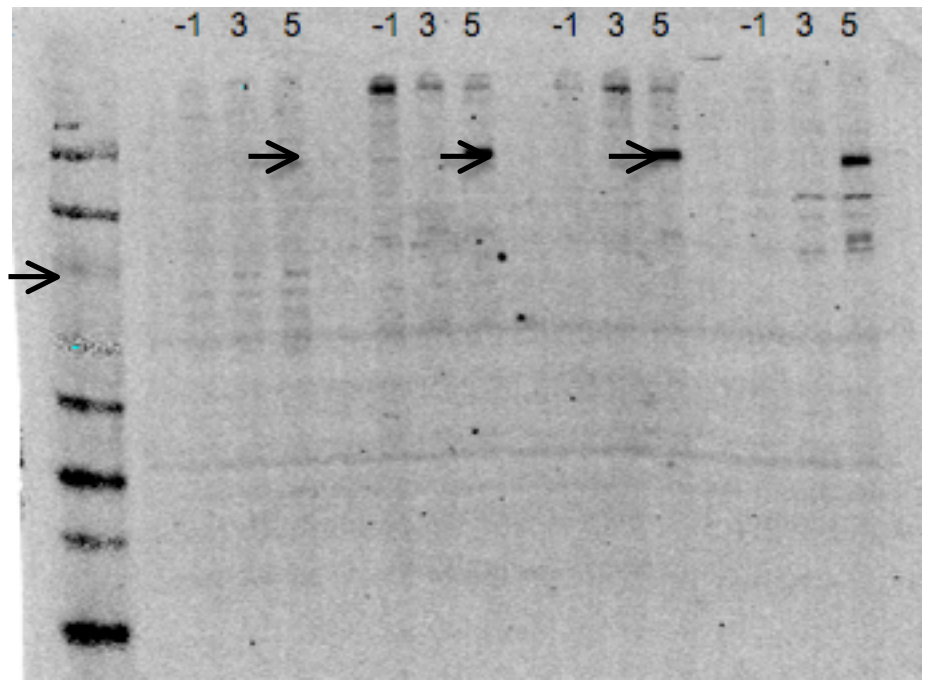


Figure 1. Autoantibody responses to TRAMP-C2 tumor pre- and post-castration. Representative Western from 4 mice. Blood samples were taken 1 week prior to castration and 3 and 5 weeks after castration. Arrows mark new autoantibody responses at 5 weeks post treatment.

Tumor control was assessed in castrated mice that were treated with anti-CD4 (GK1.5) or PBS at the time of castration and weekly thereafter as outlined in Figure 2. Blood was monitored weekly to confirm depletion of CD4⁺ T cells. In contrast to the Shionogi tumor model, CD4 depletion does not improve tumor control and although not significant, tumors are somewhat larger in the CD4-depleted mice. This may be due to the lack of CD4 help for CD8⁺ T cells during an anti-tumor response. We did find that CD8⁺ T cells from CD4-depleted mice underwent higher rates of activation induced cell death, consistent with other reports in the literature (9). As initial tumor control was not improved by CD4-depletion, we have not continued with radiation experiments in the TRAMP-C2 tumor model at this time.

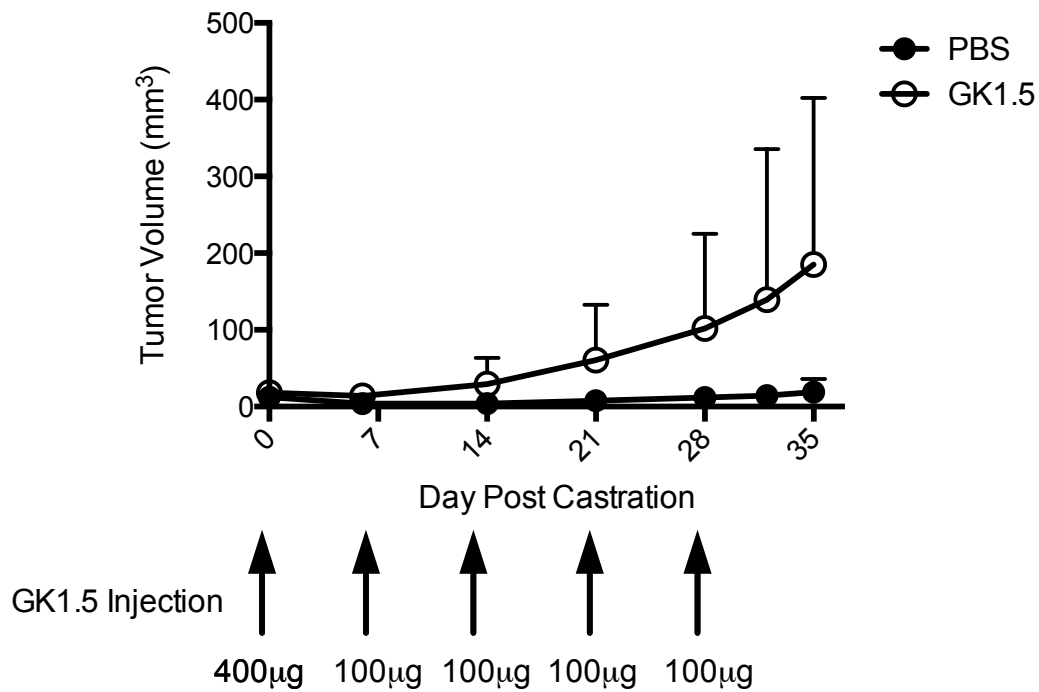


Figure 2. CD4 depletion does not improve tumor control in the TRAMP-C2 tumor model. Mice were injected with anti-CD4 at the time of castration and then given weekly maintenance doses for 4 weeks.

Task 2. To determine the role of B cell in control of TRMAP-C2 tumors.

Mice with established TRAMP-C2 tumors were given 10 mg/kg anti-CD20 (5D2) at the time of castration and one week later and tumor growth was evaluated. B cells are not detectable in the peripheral blood within 3 days of injection of anti-CD20 and is not detectable for at least 12 days after injection.

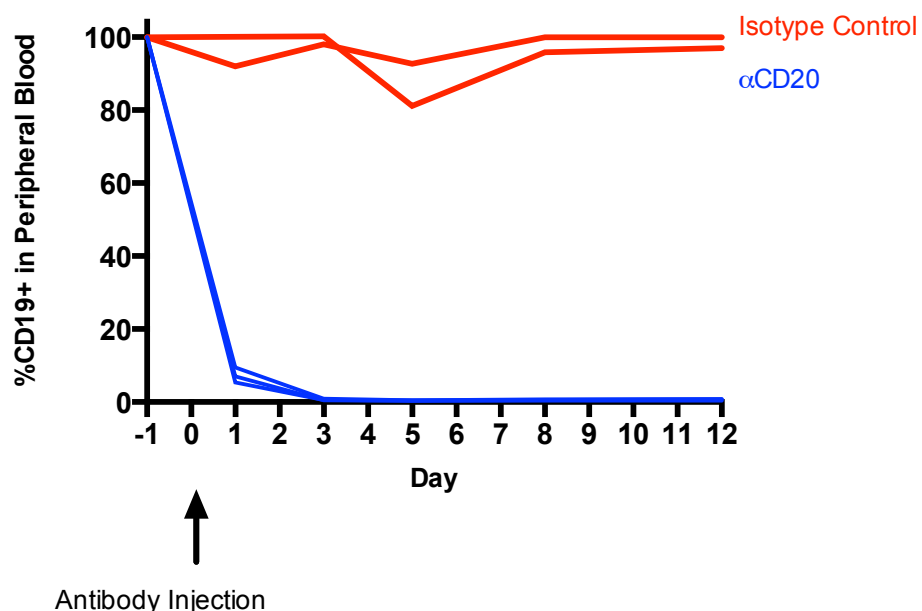


Figure 3. Kinetics of B cell depletion. The percent of CD19+ B cells were evaluated in peripheral blood 1 day prior to injection of 10 mg/kg of anti-CD20 or an isotype control. Peripheral blood taken every over 12 days to determine presence of CD19+ B cells.

Mice bearing established TRAMP-C2 tumors were castrated and either received 10 mg/kg anti-CD20 or isotype control on day 0 and day 7 post-castration. Although one mouse in the B cell depleted group grew rapidly approximately one month post castration, there appears to be increased tumor latency in the B cell depleted mice. However, overall survival was not different between the two groups. Part of the experimental design is to use B cell depletion as neo-adjuvant prior to treatment to offset any detrimental effect of ADT. We will be completing those experiments in the next 6 months.

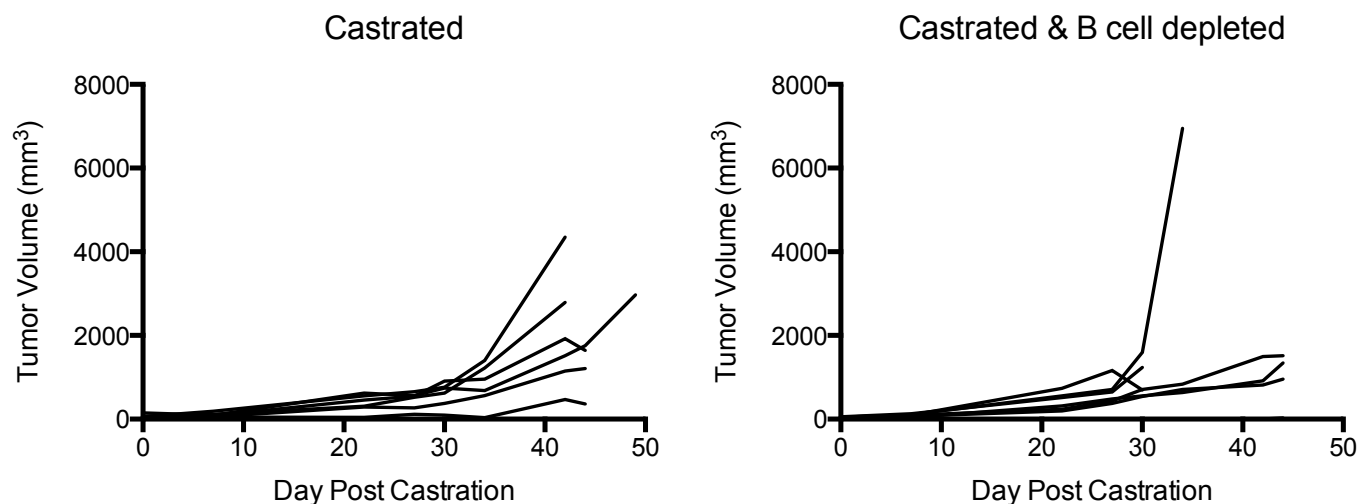


Figure 4. B cell depletion in conjunction with castration improves tumor latency. Mice bearing established TRAMP-C2 tumors were castrated and given anti-CD20 or control antibody. Tumor growth was assessed. Each line represents an individual mouse.

Task 3. To determine whether B cells affect CD8+ function in control of TRAMP-C2 tumors.

Transgenic mice specific for histone H4 (H4 TCR Tg) were brought out of cryopreservation at Jackson Labs. Once a breeding pair was available, the mice were then crossed on to Rag.SJL mice to generate congenic mice with a fixed T cell receptor. This has taken longer than expected and we have only recently generated a large enough colony and breeding pairs to perform experiments to assess the function of a tumor specific CD8⁺ T cell population in castrated mice that received B cell depletion and radiation.

Key Research Accomplishments

1. None to date.

Reportable Outcomes

1. None to date.

Conclusion

The work completed to date suggests that B cell depletion continue to be a viable potential option in the treatment of prostate cancer. We have found that CD4 depletion in the TRAMP-C2 model does not improve tumor control, and is likely necessary for the CD8+ T cell immune response to tumors. Improved survival after radiation treatment will provide more proof that an agent such as Rituximab could be combined with neoadjuvant ADT to control initial tumor growth and prevent recurrence. Experiments in the coming months will provide sufficient pre-clinical data to make a decision as to whether or not a clinical trial is warranted.

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